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Ассоциация полиморфизма генов *GDF9/G1, CAST, KRT1.2, KAP1.3* с экономически важными признаками пород овец Забайкалья

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Традиционные методы селекции овец с использованием генетических маркеров разных типов позволяют точнее идентифицировать генотипы животных, несущих высокий потенциал развития фенотипических признаков. Их реализация будет повышать прогресс преобразования стад и пород овец и позволит быстрее достичь желаемых результатов. В литературе довольно хорошо описан полиморфизм генов *GDF9/G1, CAST, KRT1.2, KAP1.3* у разных пород овец, но крайне недостаточно информации по их связи с хозяйственно ценными признаками и качеством шерсти. Цель данного исследования состояла в изучении ассоциативных связей генотипов с живой массой овец, настригом шерсти, качеством шерстяных волокон. Объект исследования – тонкорунные овцы хангильского типа забайкальской породы и полугрубошерстные овцы зугалайского типа агинской породы Забайкальского края. ПЦР – ПДРФ анализ проведен согласно описанным и апробированным методикам. Качество шерсти определено на оптическом анализаторе OFDA 2000. Установлено, что у баранов-производителей забайкальской породы с гомозиготным генотипом $CAST^{MM}$ живая масса была выше на 20,2 кг по сравнению с гетерозиготами этого гена. Бараны этой породы с генотипом $KRT1.2^{MM}$ имеют более высокий настриг шерсти по сравнению с $KRT1.2^{MN}$ на 1,2 кг. У баранов агинской породы с генотипами $CAST^{MN}$, $KAP1.3^{YY}$ была более длинная ость на 2,7 и 1,8 см, чем у животных с генотипами $CAST^{MM}$ и $KAP1.3^{XY}$ соответственно. У овцематок этой породы, наоборот, более длинная ость на 2,7 см у животных с генотипом $CAST^{MM}$ в сравнении генотипом $CAST^{MN}$. Овцематки забайкальской породы с гомозиготными генотипами $CAST^{MM}$ и $KRT1.2^{MM}$ отличаются большим углом наклона волокон (извитость) на 9,9 и 12,5 град. на 1 мм по сравнению с гетерозиготными генотипами этих генов. Исследования по выявлению ассоциативной связи генотипов с фенотипическими признаками овец следует продолжить, увеличив объем выборки.

Ключевые слова: овцы, порода, генотип, продуктивность, живая масса, настриг шерсти, качество шерстяного волокна

Association of *GDF9/G1*, *CAST*, *KRT1.2*, *KAP1.3* gene polymorphism with economically important traits of the Transbaikalia sheep breeds

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Traditional methods of sheep selection using different types of genetic markers make it possible to more accurately identify the genotypes of animals that have a high potential for the development of phenotypic traits. Their implementation will increase the progress of transformation of sheep herds and breeds and will allow the desired results to be achieved more quickly. The literature describes quite well the polymorphism of the *GDF9/G1*, *CAST*, *KRT1.2*, *KAP1.3* genes in different breeds of sheep, but there is extremely insufficient information on their relationship with economically valuable traits and wool quality. The purpose of this study was to investigate the associations of the genotypes with the live weight of sheep, wool clip, and the quality of wool fibers. The object of the study is fine-wool sheep of the Khangil type of the Zabaikalskaya breed and half-coarse-wooled sheep of the Zugalai type of the Aginskaya breed of the Transbaikal region. PCR-RFLP analysis was carried out according to the described and tested methods. The quality of wool was determined using an OFDA 2000 optical analyzer. It was found that in the rams of the Zabaikalskaya breed with the homozygous $CAST^{MM}$ genotype, the live weight was 20.2 kg higher compared with heterozygotes of this gene. Rams of this breed with the $KRT1.2^{MM}$ genotype have a higher wool clip compared to $KRT1.2^{MN}$ by 1.2 kg. Rams of the Aginskaya breed with the $CAST^{MN}$ and $KAP1.3^{YY}$ genotypes had a longer spine by 2.7 cm and 1.8 cm than the animals with the $CAST^{MM}$ and $KAP1.3^{XY}$ genotypes, respectively. In ewes of this breed, on the contrary, animals with the $CAST^{MM}$ genotype had a longer spine by 2.7 cm, compared to the $CAST^{MN}$ genotype. Zabaikalskaya breed ewes with homozygous genotypes $CAST^{MM}$ and $KRT1.2^{MM}$ are distinguished by a greater fiber inclination angle (crimp) of 9.9 and 12.5^0 on 1 mm compared to heterozygous genotypes of these genes. Research to identify the associative relationship between genotypes and phenotypic traits of sheep should be continued by increasing the sample size.

Keywords: sheep, breed, genotype, productivity, live weight, wool clip , wool fiber quality

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INTRODUCTION

Sheep breeding is a low-cost branch of animal husbandry in conditions of intensive use of pastures, including winter period. The selection of breeds is determined by the demand for this or that product in the region, and mutton has always been characterized by better sales compared to wool. Due to the increasing demand for environmentally friendly material for clothing, including wool, the market for wool has improved, and selection of sheep on the quality of wool has become more active, especially since meat productivity and wool clip closely correlate with such an indicator as live weight [1]. A good effect of increasing the selection indicators gives industrial crossing of factory breeds, the resulting crosses successfully combine meat and wool productivity [2–4].

Qualitative indicators of wool determine its technological value. Out of 40 signs characterizing wool productivity, the most valuable indicators for its processing include fineness, which has a biological nature and interrelation with other physical and technological properties, first of all, crimped fibers[1] [5].

In modern sheep breeding, new genetic methods are increasingly used, including the most promising - full genomic. Studies on Russian Merino sheep [6] have revealed five new candidate genes presumably related to fat thickness in the lumbar region.

[1]*Belik I.I.* Relationship between fineness and tortuosity of wool // Izvestiya Saint-Petersburg State Agrarian University, 2016, N 43, pp.139–144.

At the present stage of development of marker-assisted selection, a number of authors have obtained encouraging results on the use of the most tested genes associated with wool, meat productivity, growth and development of young animals. Such genes include *GDF9/G1*, *CAST*, *KRT1.2*, *KAP1.3*, which control these parameters and along with feeding and housing conditions influence their manifestation. Most of the studies of Russian and foreign scientists are devoted to the study of polymorphism and characterization of the genetic structure of individual breeds and flocks of sheep [7–12]. There is much less work on the identification of associative links of genotypes with wool and meat productivity.

The purpose of the research was to study the influence of the genotypes of *GDF9/G1*, *CAST*, *KRT1.2*, *KAP1.3* genes on live weight, wool productivity and its quality of Zabaikalskaya and Aginskaya sheep breeds.

MATERIAL AND METHODS

The researches were carried out on fine-fleece sheep of Khangil type of the Zabaikalskaya breed (ZKH, n = 100) and medium-wool breed of Zugalay type of the Aginskaya breed (AGZ, n = 100) in breeding farms of the Transbaikal Territory.

Wool quality was determined in the accredited wool laboratory of the Stavropol State Agrarian University on the optical analyzer OFDA 2000 according to the following parameters: the average value of fiber diameter (Diam, μm), the coefficient of variation of fiber diameter (*CV*, %), the comfort factor (the specific weight of wool fibers with a diameter of 30 μm and less, *CF*, %), the bending angle of wool fibers in degrees per 1 mm (Curve).

Wool from sheep was taken from the topographic area (flank). Wool clip and wool length were taken into account individually during sheep grading and shearing.

Biological material for molecular genetic studies were ear notchers of sheep (n = 200), from which genomic DNA was isolated using an extraction kit from clinical material "Ampli Prime DNA-Sorb-S-M" according to the manufacturer's prescription (AmpliSens, Moscow).

Genotyping was carried out in the laboratory of biotechnology of SibNIPTIZh SFSCA RAS according to the described and approved methods of PCR –RFLP: for *CAST*[2], *GDF9*[3], *KRT1.2*[4], *KAP1.3* [13] using restriction endonucleases produced by SibEnzyme Msp I (*CAST*, and *KRT1.2*), *BstHH* (*GDF9*), *Bse1I.* (*KAP1.3*).

RESULTS AND DISCUSSION

Live weight, like all quantitative traits, is a labile indicator, but it is a genetically determined trait. Identification of animals with desirable genotypes with maximum productivity will accelerate the breeding process and increase its efficiency. Our research has established the superiority of stud rams of the Zabaikalskaya breed with homozygous genotype $CAST^{MM}$ over heterozygous $CAST^{MN}$ by 20.2 kg (p < 0.01). Unfortunately, the differences in live weight for other genotypes of the Zabaikalskaya and medium-wool Aginskaya breeds do not reach the threshold of significance, although their level in some cases reaches 12% (see Table 1).

Other results were obtained in the studies [12]. The authors found that half-blood (1/2 Charollais + 1/2 Kalmyk fat-tailed) lambs and sheep with the genotype $\mathrm{CAST^{MN}}$ surpassed their peers with homozygous $\mathrm{CAST^{MM}}$ in live weight at the age of 8 months by 3.01 and 0.61%, respectively. Similar results [11] were obtained on young sheep of the Manych Merino

[2]*Khederzadeh S., Iranmanesh M., Motamedi-Mojdehi R.* Genetic diversity of myostatin and calpastatin genes in Zandi sheep // Journal of Livestock Science and Technologies, 2016, N 4 (1), pp. 45–52. 10.22103/JLST.2016.1381.

[3]*Hanrahan J.P., Gregan S.M., Milsant P., Mullen M., Davis G.H., Powell R., Galloway S.M.* Mutations in the genes for oocytederived growth factors GDF9 and BMP15 are associated with both in-creased ovulation rate and sterility in Cambridge and belclare ship (Ovis aries) // Biology of Reproduction, 2004, N 70 (3), pp. 900–909. DOI: 10.1095/biolreprod.103.023093.

[4]*Kumar P., Choudhary V., Kumar K.G., Bhattacharya T.K., Bhushan B., Sharma A., Mishra A.* Nucleotide sequencing and DNA polymorphism studies on IGFBP-3 gene in sheep and its comparison with cattle and buffalo // Small Ruminant Research, 2006, vol. 64, pp. 285–292. DOI: 10.1016/j.smallrumres.2005.05.018.

sheep. At the same time, a reliable superiority in live weight of the Stavropol breed with homozygous genotype $CAST^{NN}$ compared to the genotype $CAST^{MM}$ by 10.0% was established [13].

It should be noted that the most significant effect on live weight and growth energy, as studies [14] have shown, is exerted by the gene *GH*. Young sheep of the Soviet Merino breed with the genotype GH^{CT} had 5.0 and 7.6% higher birth and weaning weights than homozygous GH^{CC} individuals. Similar results on the excess of live weight of heterozygous animals over homozygotes were established in the Romanov sheep breed [15].

Wool productivity is a breeding trait that determines the competitiveness of sheep, especially fine-fleece and semifine-wool sheep. The quality of wool fiber depends on feeding conditions and hereditary information of keratin proteins *KRT1.2* and *KAP1*, which have genetic variability. Studies of domestic and foreign authors have obtained data on associative links with the most important properties of wool, live weight and growth energy [16–22].

Our research also found that rams of the Zabaikalskaya breed of Khangil type with homozygous genotypes $KRT1.2^{MM}$ have a higher wool clip compared to heterozygous $KRT1.2^{MN}$ by 1.2 kg ($p < 0.01$). No significant differences were found for the other genotypes of rams and for all genotypes of ewe lambs (see Table 2).

In ewes and rams of the Aginskaya breed of Zugalai type, depending on genotypes, no significant differences in wool shearing were revealed (see Table 3).

In rams of the Aginskaya breed the superiority in awn length with genotypes $CAST^{MN}$ over the homozygous genotype by 2.7 cm and with genotype $KAP1.3^{YY}$ over genotype $KAP1.3^{XY}$ by 1.8 cm, respectively ($p < 0.05$). In ewe lambs, on the contrary, a longer awn (by 2.7 cm) was detected in the animals with homozygous $CAST^{MM}$ genotype than in heterozygous $CAST^{MN}$ ($p < 0.01$).

When analyzing the technological properties of wool (fineness, variability, slope angle and comfort factor), no significant differences between rams of the Zabaikalskaya breed of Khangil type depending on the genotype were revealed (see Table 4). However, in further breeding work attention should be paid to animals with the genotype $CAST^{MN}$, which have thinner wool (by 1.6–1.9 microns) and higher, comfort factor – by 2.8–3.7 abs.%.

Ewes of the Zabaikalskaya breed with homozygous genotypes $CAST^{MM}$ and $KRT1.2^{MM}$ are distinguished by a greater angle of fiber inclination (tortuosity) (by 9.9 and 12.5 degrees

Табл. 1. Живая масса овец с учетом генотипов, кг
Table 1. Live weight of sheep taking into account the genotypes, kg

Genotype	Breed, type							
	ZKH				AGZ			
	n	♂	*n*	♀	*n*	♂	*n*	♀
$GDF9/GI^{AG}$	–	–	5	58,0 ± 2,60	4	76,8 ± 4,70	6	57,3 ± 0,92
$GDF9/GI^{GG}$	29	90,7 ± 3,5	66	58,2 ± 0,86	27	72,8 ± 1,78	63	56,6 ± 0,47
$CAST^{MM}$	25	93,2 ± 3,3	57	58,4 ± 0,95	28	73,5 ± 1,78	58	56,2 ± 0,42
$CAST^{MN}$	4	73,0 ± 6,2[2]	13	57,4 ± 1,63	3	71,3 ± 4,91	10	59,3 ± 1,56
$KRT1.2^{MM}$	25	92,1 ± 3,5	49	58,2 ± 0,90	25	73,7 ± 1,80	62	56,7 ± 0,48
$KRT1.2^{MN}$	4	80,0 ± 7,5	20	56,9 ± 1,40	6	71,7 ± 4,34	7	55,9 ± 0,94
$KAP1.3^{XX}$	20	90,9 ± 4,0	41	56,9 ± 1,00	7	73,7 ± 4,27	25	56,8 ± 0,75
$KAP1.3^{XY}$	9	89,3 ± 6,0	28	59,9 ± 1,50	18	74,5 ± 2,07	25	56,2 ± 0,71
$KAP1.3^{YY}$	–	–	–	–	4	66,5 ± 2,53	19	56,9 ± 0,86
On average	29	90,4 ± 17,52	71	58,1 ± 6,91	31	73,3 ± 9,20	69	56,6 ± 3,64

Табл. 2. Шерстная продуктивность овец ЗТХ с учетом генотипов
Table 2. Wool productivity of ZFKh sheep taking into account the genotypes

Genotype	Wool clip, kg				Wool clip, cm	
	n	♂	*n*	♀	♂	♀
GDF9/GI^{AG}	–	–	5	4,8 ± 0,17	–	10,2 ± 0,58
GDF9/GI^{GG}	29	10,5 ± 0,22	66	4,7 ± 0,06	10,9 ± 0,20	9,8 ± 0,09
CAST^{MM}	25	10,6 ± 0,22	57	4,8 ± 0,07	10,8 ± 0,22	9,9 ± 0,10
CAST^{MN}	4	9,8 ± 0,70	13	4,7 ± 0,08	11,1 ± 0,43	9,7 ± 0,24
KRT1.2^{MM}	25	10,6 ± 0,23	49	4,7 ± 0,07	10,9 ± 0,22	9,9 ± 0,11
KRT1.2^{MN}	4	9,4 ± 0,36^{2}	20	4,8 ± 0,09	10,3 ± 0,43	9,7 ± 0,18
KAP1.3^{XX}	20	10,5 ± 0,26	41	4,7 ± 0,07	10,9 ± 0,23	9,9 ± 0,13
KAP1.3^{XY}	9	10,3 ± 0,42	28	4,8 ± 0,10	10,7 ± 0,42	9,8 ± 0,15
On average	29	10,4 ± 1,16	71	4,7 ± 0,47	10,8 ± 1,07	9,8 ± 0,80

Табл. 3. Шерстная продуктивность овец АГЗ с учетом генотипов
Table 3. Wool productivity of AGZ sheep taking into account the genotypes

Genotype	Wool clip, kg				Wool clip, cm			
					down		awn	
	n	♂	*n*	♀	♂	♀	♂	♀
GDF9/GI^{AG}	4	3,7 ± 0,15	6	2,6 ± 0,16	7,5 ± 0,87	6,7 ± 0,71	19,0 ± 1,35	16,8 ± 1,62
GDF9/GI^{GG}	27	3,6 ± 0,05	63	2,8 ± 0,03	7,7 ± 0,28	6,7 ± 0,16	19,3 ± 0,49	17,9 ± 0,37
CAST^{MM}	28	3,6 ± 0,05	58	2,8 ± 0,04	7,6 ± 0,28	6,7 ± 0,18	19,0 ± 0,46^{1}	18,1 ± 0,39
CAST^{MN}	3	3,6 ± 0,20	10	2,8 ± 0,08	8,3 ± 0,88	6,7 ± 0,33	21,7 ± 1,20	15,9 ± 0,85^{2}
KRT1.2^{MM}	25	3,6 ± 0,06	62	2,8 ± 0,04	7,7 ± 0,32	6,7 ± 0,17	19,3 ± 0,52	17,8 ± 0,40
KRT1.2^{MN}	6	3,5 ± 0,12	7	2,8 ± 0,10	7,8 ± 0,31	6,7 ± 0,52	19,2 ± 0,98	18,3 ± 0,71
KAP1.3^{XX}	7	3,6 ± 0,14	25	2,8 ± 0,06	7,9 ± 0,67	6,7 ± 0,26	18,9 ± 1,20	17,6 ± 0,61
KAP1.3^{XY}	18	3,6 ± 0,07	25	2,8 ± 0,05	7,6 ± 0,31	6,8 ± 0,25	19,2 ± 0,57^{1}	17,9 ± 0,48
KAP1.3^{YY}	4	3,5 ± 0,04	19	2,7 ± 0,06	8,3 ± 0,75	6,7 ± 0,34	21,0 ± 0,58	17,9 ± 0,86
On average	31	3,6 ± 0,28	69	2,8 ± 0,28	8,0 ± 2,23	6,7 ± 1,33	19,3 ± 2,52	17,8 ± 3,01

per 1 mm) ($p < 0.05$) compared to heterozygous genotypes (see Table 5).

Despite the considerable amount of information on the genetic structure of sheep of different breeds on *KRT1.2* and *KAP1.3* genes, there are few data on the relationship with wool productivity and quality indicators of wool. According to researchers [13, 19], the specificity of allele and genotype frequency distribution for *KRT1.2* and *KAP1.3* genes in wool (fine and semi-fine) and meat breeds of sheep suggests that selection for wool productivity increases the frequency of genotypes associated with these indicators. Our previous studies [20] found that the frequency of the *KRT1.2*^{MM} genotype in the Kulunda fine-fleece breed is 95.5%, whereas in the West Si-

Табл. 4. Показатели качества шерсти баранов ЗТХ с учетом генотипов
Table 4. Indicators of the quality of wool from ZFKh rams taking into account the genotypes

Genotype	*n*	Indicator			
		Diam, µm	*CV*, %	Curve, deg. per 1 mm	*CF*, %
GDF9/GI	27	23,5 ± 0,48	19,8 ± 0,39	69,6 ± 1,92	90,7 ± 1,51
CAST^{MM}	25	23,7 ± 0,45	19,8 ± 0,39	70,3 ± 2,02	90,0 ± 1,56
CAST^{MN}	4	21,8 ± 2,06	20,7 ± 0,76	67,3 ± 3,78	93,5 ± 5,55
KRT1.2^{MM}	25	23,5 ± 0,49	19,7 ± 0,40	70,2 ± 1,99	90,6 ± 1,61
KRT1.2^{MN}	4	23,4 ± 1,96	20,7 ± 0,76	67,9 ± 4,74	89,8 ± 5,04
KAP1.3^{XX}	20	23,5 ± 0,58	19,9 ± 0,41	69,2 ± 2,21	90,1 ± 1,85
KAP1.3^{XY}	9	23,6 ± 1,04	20,3 ± 0,72	73,1 ± 3,16	90,4 ± 3,01

berian meat breed it is only 32.8%, which may serve as indirect evidence of the association of this genotype with wool productivity, for which selection in the Kulunda fine-fleece breed is intensive.

To date, unambiguous results on the relationship between genotypes and economically valuable indicators have not been revealed. Thus, the study using *SNPs* for 84 predicted genes *KAP* and *KRT* did not allow to determine their significant influence on wool shearing, diameter and fiber tortuosity[5]. However, the search for genetic markers of wool phenotypic traits continues. Mutation in the *FGF-5* gene has been identified, which may influence the wool productivity of sheep [21]. In this case, hair growth can be increased by changing the expression of this gene.

Candidate genes associated with breast depth and width, meat quality[6] [22] are searched by the method of full genomic analysis. At the same time, the authors of the cited works indicate the need for further research to confirm the results obtained.

Табл. 5. Показатели качества шерсти овцематок ЗТХ с учетом генотипов
Table 5. Indicators of the quality of wool of ZFKh ewes taking into account the genotypes

Genotype	*n*	Indicator			
		Diam, µm	*CV*, %	Curve, deg. per 1 mm	*CF*, %
GDF9/GI	19	23,0 ± 0,38	24,0 ± 0,58	70,5 ± 3,17	89,5 ± 1,19
CAST^{MM}	16	23,2 ± 0,45	23,9 ± 0,68	73,2 ± 3,54	88,9 ± 1,36
CAST^{MN}	5	23,3 ± 0,72	24,3 ± 0,92	63,3 ± 3,41[1]	89,1 ± 2,35
KRT1.2^{MM}	16	23,0 ± 0,46	23,7 ± 0,67	73,8 ± 3,26	89,8 ± 1,27
KRT1.2^{MN}	5	23,8 ± 0,57	24,9 ± 0,84	61,3 ± 4,77[1]	86,3 ± 2,46
KAP1.3^{XX}	11	23,5 ± 0,54	24,0 ± 0,62	67,4 ± 4,15	87,9 ± 1,48
KAP1.3^{XY}	10	22,8 ± 0,51	24,0 ± 0,98	74,7 ± 4,00	90,2 ± 1,77

[5] *Phuaa S.H., Scobieb D.R., O'Connell D., Henrya H., Doddsa K.G., Brauninga R., Clerensb S.* Preliminary linkage studies in sheep of keratin and keratin-associated protein genes with fleece weight, wool fibre diameter and fibre curvature // Proc. N. Z. Soc. Anim. Prod, 2015, vol. 75, pp. 101–105.

[6] *Yatsyk O.A., Krivoruchko A.Yu.* Full genomic search for associations of single nucleotide substitutions with breast depth in sheep of the Russian meat merino breed // Collection of scientific papers of the Krasnodar Scientific Center for Animal Science and Veterinary Medicine, 2021, N 1, pp. 143–146.

CONCLUSIONS

1. The genes *GDF9/G1*, *CAST*, *KRT1.2*, *KAP1.3* with the analysis of allele and genotype frequencies, homo- and heterozygosity and other population genetic parameters in different breeds are rather well tested in sheep breeding. At the same time, there are very few studies on the relationship of genotypes *CAST* with meat productivity, genotypes *KRT1.2* and *KAP1.3* with qualitative and quantitative indicators of wool, *GDF9/G1* - growth energy of young animals, which may be due to the difficulty of keeping breeding records, unlike cattle, and insufficient sample size.

2. When studying the relationship of genotypes of *GDF9/G1*, *CAST*, *KRT1.2*, *KAP1.3* genes with phenotypic traits in the comparative evaluation of the two leading sheep breeds of Transbaikalia, contradictory data were obtained. The priority of $CAST^{MM}$ genotype by live weight in stud rams of the Zabaikalskaya breed is not confirmed on ewes of this breed and in the Aginskaya breed. The $KRT1.2^{MM}$ genotype was attributed to the desirable genotype in stud rams of the ZHT breed, the best genotypes for this indicator were not revealed in ewe lambs. In the Aginskaya breed in rams longer awn is associated with genotypes $CAST^{MN}$ and $KAP1.3^{YY}$, while in ewes of this breed, on the contrary, longer awn is revealed in the owners of genotype $CAST^{MM}$. Good wool crimp was characterized by sheep sows of the Zabaikalskaya breed with homozygous genotypes $CAST^{MM}$ and $KRT1.2^{MM}$.

3. The identified differences can be considered as preliminary, requiring the accumulation of more observations, preferably in different herds and breeds.

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